Original research article

Genomic insights in Andhra Pradesh Covid-19 Saga: Districts wise Variant Analysis for 2023

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Abstract

Background: The Covid-19 pandemic, as the inaugural pandemic in the Post Genomic era, has greatly benefited from the advancements in Genomics, leading to enhanced scientific comprehension and improved public health measures. This study focuses on the Whole Genome Sequencing of 894 Covid positive samples collected from various districts of Andhra Pradesh. The sequencing was conducted at the Centre for Cellular and Molecular Biology, Siddhartha Medical College Vijayawada, an INSACOG laboratory.

Methods: Retrospective observational study carried out at CCMB LAB, INSACOG, Siddhartha Medical College, Vijayawada from January 2023 to August 2023. This study focuses on the analysis of Covid-19 positive samples collected from various districts in Andhra Pradesh, India. The samples were processed using RNA extraction and whole genome sequencing techniques, specifically utilizing Minon MK1C. The obtained genomic data was then analyzed using software tools such as Next Clade and Pangolin to identify any Variants of Concern (VOC). This paper presents the findings of a study that were shared with IHIP, IBDC and GISAID portals, as well as communicated to INSACOG.

Results: A total of 894 samples, confirmed positive for Covid-19, originating from various districts in Andhra Pradesh, were forwarded to the Center for Cellular and Molecular Biology (CCMB), SMC LAB for Whole Genome Sequencing (WGS) analysis. A comparative analysis was conducted on a cohort of Omicron mutants, comprising the total sample size. The present study reports the identification of several mutants, predominantly XBB.1.16 and XBB.2, along with their respective subvariants. Additionally, a smaller number of BA.2 and BA.5 mutants were also identified.

Conclusion: In conclusion, to effectively combat any pandemic, it is imperative to employ a daily determination of variant of concern (VOC) through whole genome sequencing (WGS) analysis. This approach should be applied to all samples testing positive for Covid-19 across all districts within the state. By implementing this comprehensive strategy, we can enhance our ability to monitor and mitigate the spread of emerging viral variants, thereby safeguarding public health. In conclusion, implementing measures to restrict the spread of the VOC variant and prioritizing public health through the limitation of transportation to affected areas will greatly assist the government in effectively managing the situation.

Keywords: WGS, VOC, MINION, MKIC, Next Clade, Pangolin, IBDC, GISAID, INSACOG

Introduction

It was more than 100 years since the 1918 influenza outbreak killed at least fifty million people world wide1. Now we have witnessed another pandemic. Since December of 2019, the 2019 novel coronavirus disease (COVID-19) has spread rapidly throughout the world, resulting in millions of confirmed cases and hundreds of thousands of deaths in less than 6 months (Bootsoma *et al*., 2007). The disease was caused by the infection of a novel enveloped RNA beta-coronavirus that has been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the seventh coronavirus species that causes respiratory disease in humans (Kang *et al*., 2020). The virus causes serious respiratory illnesses such as pneumonia, lung failure, and even death. Continuing epidemiological and Molecular biological study to better understand, treat and prevent COVID-19 are urgently needed. A characteristic feature of many

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human infections is that only a proportion of exposed individuals develop clinical disease and for the infected persons, severity varies from person to person7. In the COVID-19 outbreak, a high level of interindividual variability was observed in terms of disease severity and symptomatic presentation (Rgensen *et al*., 2023).

Whole genome sequencing informs evidence-based public health decisions by providing crucial data for disease transmission, new outbreak detection, as demonstrated by HIV3, tuberculous, Ebola 5, 6 and Zika 7 outbreaks. In the immediate response to COVID-19, several studies have demonstrated that genomic surveillance outcomes were not only comparable to epidemiological contact tracing but also capable of tracing previously unknown linked transmissions (Kang *et al*., 2020). Genomic investigation informed the public health decisions to prevent further spread of SARS-CoV-2, including travel restrictions and stay-at home orders in response to the identification of travel-related clusters and local clusters 10. Rapid SARS-CoV-2 whole genome sequencing is therefore an essential public health measure.

Aims and Objectives

1. To determine the VOC, Day wise in the State of Andhra Pradesh.
2. To know the VOC in individual Districts of Andhra Pradesh.
3. To help the State Government and National administration to put a check on the Pandemic.
4. To help check the areas to be barcoded.
5. To check the virulence of the mutants identified.

Materials & Methods

Sample collection & receiving

- Positive samples are collected from all over AP and sent to CCMB lab-SMC, VJA.
- Once the samples are received quality check of the sample is done, Which includes the following.

Inclusion criteria

- Triple packing-sample should be triple packed with no leakage.
- Cold-chain maintenance-samples must be brought with dry ice so that the temperature is maintained.
- Sample quantity.
- Standard Conditions of the outer package.
- CT value with Covid RTPCR less than 25.

Sample rejection/exclusion criteria

If the samples received are not following the above conditions those samples are rejected.

Even if the Covid positive samples are tested by Rapid tests or Trunat they are rejected

- After the quality check, samples received are entered in receiving register, ICMR forms are collected and samples are stored in -80C freezer.
- Later on all these samples are pooled until they are 96 in number then further processed to Extraction.
- **Sample pooling:** All the samples are stored in -80°C and pooled until they are 96 in number. Once the count is 96 then they are prepared for extraction. Samples are labeled and given an Id for further identification.

Extraction: spin-column based extraction.

Kit Used-GENES 2 ME (Reagent required like carrier-RNA, proteinase K, lyses buffer, wash buffer 1,2 and elution buffer are provided in the kit).

- In 1.5ml low binding centrifuge tube, add 400µl of lysis buffer, 10µl of proteinase K and 1µl carrier RNA.
- Transfer 200 µl of sample and mix the sample by vortex ting for15 seconds. Leave the sample in room temperature for 5-10 min.
- Take out a new viral mini column and transfer the sample to the column, centrifuge at 10,000×g for 30-60 seconds.
- Discard the filtrate and place the column back into the collection tube. Add 500 µl of wash buffer to the column, centrifuge at 10,000×g for 30-60 seconds. Repeat the step again.
- Discard the filtrate and place the column back into the collection tube. Centrifuge at 13,000×g for 3 minutes to dry the column.
- Transfer the column to a new 1.5 ml centrifuge tube.
- Add 30µl-50 µl elution buffers to the center of the membrane of the column. Centrifuge at 13,000×g for 1 minute.
- Discard the column and store the RNA at -80 °C.
- Once the RNA is ready it is further used to process sequencing.
- Covid RNA is sequenced in 2different methods i.e.,

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1. Rapid bar-coding and mid night RT PCR expansion method.
2. ARTIC v3 Protocol with Native Bar-coding kit.

Rapid barcoding and mid night RT PCR expansion method
Materials
• Mid night RT-PCR expansion kit (EXP-MRT001) • Nuclease-free water • Freshly-prepared 80% ethanol in nuclease-free water • 1.5 ml Eppendorf DNA LowBind tubes • 2 ml Eppendorf DNA LowBind tubes • 5 ml Eppendorf DNA LowBind tubes • Eppendorf twin.tec® PCR plate 96 LowBind, semi-skirted with PCR seals • Qubit dsDNA HS Assay Kit • Qubit™ Assay Tubes • Rapid Barcoding Kit 96 (SQK-RBK110.96) • Flow Cell Wash Kit (EXP-WSH004)

Procedure: • Library preparation using mid night RT PCR expansion kit initiates with defrosting of Lunascript super mix. And followed by the kit primer both A and B. • The rapid barcodes from barcoded attached plate were used only after by spin. • Washed the amplicons with 80% ethanol. For the quantification of DNA Qubit dsDNA HS Assay reagent was used by Qubit fluorometer plate reader (or equivalent for QC check). • Finally, 80ng of final library was prepared with the combination of Rapid Adaptor, Sequencing Buffer-Loading beads.

ARTIC v3 Protocol with Native Barcoding kit
Materials: • Super SCRIPT-IV • AMPure XP beads • NEB-NEXT Q5 master mix • NEB-Next ULTRA-II End prep kit • NEB-Next ULTRA-II ligation kit • NEB-Next ULTRA-II enhancer kit • AMX-II • Sequencing buffer • Loading beads • Nuclease-free water • Freshly-prepared 80% ethanol in nuclease-free water • 1.5 ml Eppendorf DNA LowBind tubes • 2 ml Eppendorf DNA LowBind tubes • Eppendorf twin.tec® PCR plate 96 LowBind, semi-skirted with PCR seals • Qubit dsDNA HS Assay Kit • Qubit™ Assay Tubes.

Procedure: • For cDNA preparation, Random hexamers and 10mM dNTPs, 5X SSIV buffer, 0.1M DTT, RNase inhibitor, SSIV RT from Superscript First strand synthesis kit was used. • Slowly mix with the pipette and give a short spin. • The primers were used by ARTIC network (https://artic.network/ncov-2019) • To the purified amplicons the Native barcodes were tagged by the help of ultra-II ligation kit. •Qubit dsDNA HS Assay reagent was used to quantify the barcoded purified DNA in Qubit fluorometer plate reader (or equivalent for QC check). •Later on, adaptor ligation was made by using 160ng of purified barcoded DNA with the combination of NEB-Next Ultra ligation kit as compatible • The final wash was made by using short fragment buffer (SFB) rather than 80% ethanol in this step. • The final loading library was prepared with the 80ng of purified library, sequencing buffer, loading beads by sum up the total volume into 70 µl for the addition of flow cell.

- Before the library is loaded in the flow-cell, flow cell is washed then hardware check and pore check done.
- Once the pores are in required amounts, library is loaded.
- Then program setup is done. Run might take up to 18- 72hrs to get the raw files-fastq and fasta5.
- These fastq files are further processed by using bioinformatics-pipeline then data is analyzed in following steps-Running Artic-Rampart, Adapter Trimming Using Porechop, Alignment, and Variant Calling & Consensus Generation.
- Once the consensus is finalized, clade and lineage assignment can be done by using Nextclade and pangolin respectively.
- After clade and lineage assignment results are uploaded to IBDC, IHIP and GISAID portals and reported to INSACOG.

Results

Table 1

S. No.	No. of Samples	Area
1.	2	Anakapalli
2.	9	Ananthapuram
3.	2	Bapatla
4.	3	Chittor
5.	86	East Godavari
6.	330	Eluru
7.	61	GUNTUR
8.	65	Krishna
9	12	Krishna
10	5	Kurnool
11	4	Nellore
12	285	NTR

13	1	Palnadu
14	7	Prakasham
15	1	Tirupati
16	6	Visakhapatnam
17	14	West Godavari
18	1	Y.S. R
	Total	894

Table. 1: In the period from January 2023 to August 2023, a total of 894 samples testing positive for Covid were received. Among these samples, the highest number was reported from ELURU, NTR district, and East Godavari Districts.

Table 2

S. No.	Area	No. of Samples	Most Frequent Variants
1.	Anakapalli	2	XBB.1.16
2.	Ananthapuram	9	XBB.1.16, XBB.1.16.1, Indeterminate
3.	Bapatla	2	XBB.1.16
4.	Chittor	3	XBB.1, XBB.2.3
5.	East Godavari	86	XBB.1.16, XBB.1.16.1, XBB.19, XBB.3, XBB.2.3, XBB.3, Indeterminate.
6.	Eluru	330	XBB.1, XBB.1.16, XBB.1.16.1, XBB.1.16.8, XBB.1.5, XBB.1.9.1, XBB.1.9.1, Indeterminate.
7.	Guntur	61	BA.2, BF.11, CH.1.1, XBB.1, XBB.1.16, XBB.1.16.1, XBB.1.5, XBB.1.9, XBB.2.23, XBB.2.3, Indeterminate.
8.	Krishna	77	BA.2, XBB.1.16, XBB.1.16.1, XBB.1.5, XBB.2.3, Indeterminate.
9.	Kurnool	5	XBB.1.16, Indeterminate.
10.	Nellore	4	XBB.1.16, XBB.2.3
11.	NTR	285	BA.2, BA.2.75, XBB.1, XBB.1.16, XBB.1.16.1, XBB.1.5, XBB.1.5.18, XBB.1.8, XBB.1.9.1, XBB.2, XBB.2.3, XBB.2.3.2, Indeterminate.
12.	Palnadu	1	XBB.1.16.1
13.	Prakasham	7	XBB.1.16.1, XBB.1.5
14.	Tirupati	1	Indeterminate
15.	Visakhapatnam	6	BA.5, BQ.1.1, XBB.1, XBB.1.5
16.	West Godavari	14	XBB.1, XBB.1.16, XBB.2.3, XBB.2.3.4, XBB.1.9.1, Indeterminate
17.	Y.S. R	1	Indeterminate
	Total	894	

Table 2: The following report presents the Variants of Concern (VOCs) that have been identified from various districts. The majority of the Variants of Concern (VOCs) detected in this study were found to be mutants of OMICRON-XBB and its subvariants, as well as BA2 and BA.5.

Table 3

S. No.	Month	No. of Samples	Most Frequent Variant
1.	January	9	BA.5, BF.11, CH.1.1, XBB.1, XBB.1.5
2.	February	7	XBB.1.5, XBB.2.3, XBB.3
3.	March	57	BA.2, BA.2.75, XBB.1, XBB.1.16, XBB.1.9, XBB.2, XBB.2.3, Indeterminate
4.	April	335	BA.2, BA.2.75, XBB.1, XBB.1.16, XBB.1.5, XBB.1.5.18, XBB.1.8, XBB.1.9.1, XBB.2.23, XBB.23.
5.	May	477	XBB.1.6, XBB.1.16.1, XBB.2, XBB.2.3.2, XBB.2.3.
6.	June	6	XBB.1.16, XBB.1.16.8, XBB.2.3.4.
7.	August	3	XBB.1.5, XBB.1.9.1

Table 3: The provided data illustrates the distribution of VOC (Variant of Concern) on a monthly basis. During the months of January, February, March, and April, the variant of concern known was BA.2 and XBB.1. During, the later months, there was a noticeable change in the variant, which became a milder version of Omicron, XBB.1 and XBB.2 mutants. The statement suggests that there was a previous occurrence or event, and the current situation being referred to is considered the third wave of that series.

Table 4

Variant	Count of Variant
BA.2	7
BA.2.75	3
BA.5	1
BF.11	2
BQ.1.1	1

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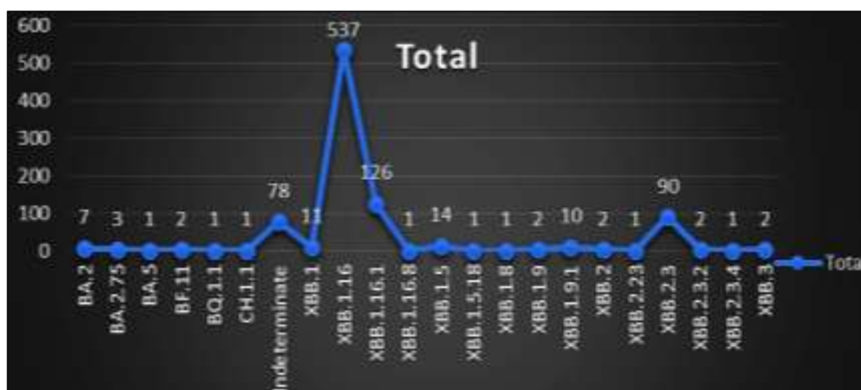
CH.1.1	1
Indeterminate	78
XBB.1	11
XBB.1.16	537
XBB.1.16.1	126
XBB.1.16.8	1
XBB.1.5	14
XBB.1.5.18	1
XBB.1.8	1
XBB.1.9	2
XBB.1.9.1	10
XBB.2	2
XBB.2.23	1
XBB.2.3	90
XBB.2.3.2	2
XBB.2.3.4	1
XBB.3	2
Grand Total	894

Table 4: The number of the most often identified Omicron Mutant is displayed in this table. Following, XBB.16 and XBB.16.1 in order of maximum isolation was XBB.2.3.



Discussion

It is essential to identify VOCs in order to stop the pandemic from spreading. In the INSACOG laboratory at CCMB LAB, on the Siddhartha Medical College campus, Vijayawada, the whole genomes of Covid positive samples with CT values less than 25 from various districts in the State of Andhra Pradesh were sequenced in order to discover the variant of concern.

As mentioned in the materials and methods above, Covid positive samples were extracted for RNA and sequenced using Oxford Nanopore reagents in Minion MKIC. Bioinformatic pipeline was used to process Fasta and Fastaq files. Using the Next Clade and Pangolin pipelines, VOC was identified. VOC was reported to INSACOG through the IBDC portal and to the State via the IHIP portal. The files were all concurrently uploaded to GISAID.

The district authorities, state authorities and National authorities were all benefited greatly from the reporting of different variants for concern because it helped them concentrate on public health facilities and also aid to stop the epidemic. Sequencing was done on a total of 894 Covid positive samples. WGS was performed on 330 Covid positive samples with CT values less than 25 from Eluru district, including 285 samples from NTR district, 86 samples from East Godavari district, and 77 samples from Krishna district. The proximity of these places to the sequencing facility at the Siddhartha Medical College in Vijayawada is likely the cause of the high sample size from these areas. The laboratory received these samples between January 2023 and August 2023. (TABLE 1) Omicron variations, which were the leading variant globally in 2023 during the corresponding months of the year, were detected in all samples from districts. In the majority of the districts, Omicron XBB.1 and XBB.1.16, XBB.1.5, and XBB.2 variants were found. The most prevalent variations found are shown in Table 2.

According to Table 3, the Omicron variants BA.2 and XXB.1 were more prevalent in the months of January, February, March, and April. A small number of BA.5 mutations were discovered and immediately notified to INSACOG and State Officials.

The VOC changed to a milder variant of Omicron XXB.1.6, XBB.1.9, XBB.2, and XBB.2.3 in the months of May, June, and August. Since June 2023, fewer samples have tested positive for Covid, however the predominant Omicron mutant in these samples is still an XBB variety with moderate mutations.

The number of distinct Omicron mutants identified by WGS in 2023 is shown in Table 4 along with a

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graph. The most prevalent version found was XBB.1.16. The following most common variant was XBB.1.16.1. According to the graph, XBB.2 was in third place for highest frequency. Samples with uncertain results were produced by faulty samples and lengthy transportation delays.
Since our lab is a hub lab, the Bioinformatic Pipeline at the Centre for Cellular Molecular Biology in Hyderabad has verified our results. The State Government and INSACOG have taken control of the CCMB lab SMC in Vijayawada.

Conclusion
In conclusion, to effectively combat any pandemic, it is imperative to employ a daily determination of variant of concern (VOC) through whole genome sequencing (WGS) analysis. This approach should be applied to all samples testing positive for Covid-19 across all districts within the state. By implementing this comprehensive strategy, we can enhance our ability to monitor and mitigate the spread of emerging viral variants, thereby safeguarding public health. In conclusion, implementing measures to restrict the spread of the VOC variant and prioritizing public health through the limitation of transportation to affected areas will greatly assist the government in effectively managing the situation.

Conflict of interest
None

Funding
Nil

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